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86. Hiromu Hitomi, Satoshi Horii, Takeshi Yamaguchi, and Akira Miyake: Chemistry of Zygomycin-A. I.*1,2 Degradative Studies; Pseudoneamine and D-Ribose.

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In the previous report[1] it was mentioned that antibiotic No. 45449A belongs to the neomycin group but differs from kanamycin, neomycin, or hydroxymycin,[2] and closely resembles paromomycin[3] reported in the midst of the present work.

Further studies[4] revealed that antibiotic No. 45449A was different from paromomycin in the diaminohexose moiety, and therefore the name zygomycin-A was assigned to this antibiotic.

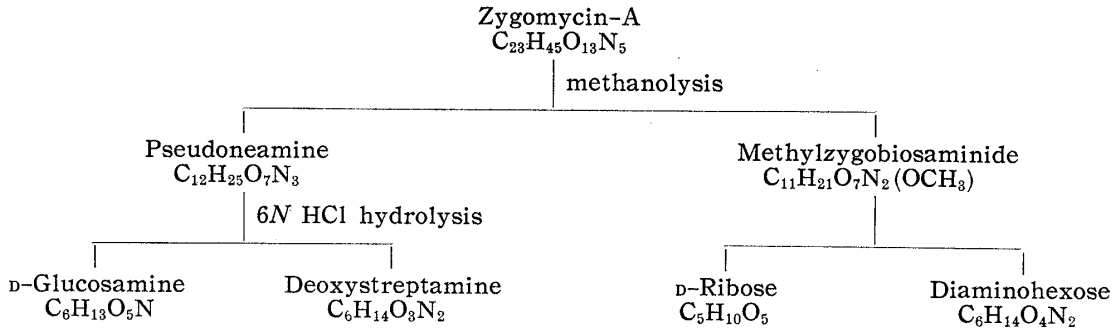
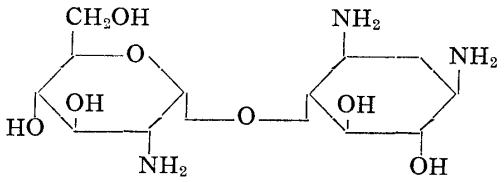


Chart 1. Isolation Procedure for the Degradation Products



(I) Pseudoneamine

In the present paper are reported the isolation and identification of pseudoneamine and D-ribose, which were separated from the degradation products of zygomycin-A.

Methanolysis of zygomycin-A hydrochloride in 0.3*N* methanolic hydrogen chloride for 3 hours gave a crystalline hydrochloride (I), $C_{12}H_{25}O_7N_3 \cdot 3HCl$. Hydrolysis of (I) by refluxing with 6*N* hydrochloric acid for 1.5 hours gave two compounds, (II), $C_6H_{13}O_5N$ and (III), $C_6H_{14}O_3N_2$, which were purified by fractional crystallization or by ion exchanger chromatography. (II) was assumed to be an amino-sugar since it showed positive Elson-Morgan, Ninhydrin, and Fehling tests. Identity of (II) with D-glucosamine was established by the measurement of infrared spectrum, X-ray diffraction pattern, and specific rotation, as well as by mixed melting point determination.

(III) was positive to the Ninhydrin reaction but negative to the Elson-Morgan and Fehling tests. This optically inactive compound proved to be identical with deoxystrepta-

*1 This constitutes Part XXVI of a series entitled "Studies on Antibiotics" by Sueo Tatsuoka.

*2 This paper was presented at the 116th and 118th Meetings of the Japan Antibiotic Research Association, September 18, 1959, and January 22, 1960.

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2) G. Hagemann, G. Nominé, L. Pénasse: Ann. pharm. franç., **16**, 585 (1958).
3) T.H. Haskel, J.C. French, Q.R. Bartz: J. Am. Chem. Soc., **81**, 3480 (1959).
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mine[5,6] produced from neomycin and kanamycin, since its infrared absorption spectrum and X-ray diffraction pattern were the same as those of an authentic sample and it showed no depression in melting point when mixed with an authentic sample. N,N′,N″-Triacetyl derivative of (I), prepared by the method of Roseman and Ludowieg,[7] consumed two moles of periodate in acetate buffer of pH 4.2, producing no formic acid.

These data, coupled with its infrared spectrum, suggested that (I) was identical with pseudoneamine produced from hydroxymycin and paromomycin. Fig. 1 shows the infrared absorption spectrum of pseudoneamine trihydrochloride.

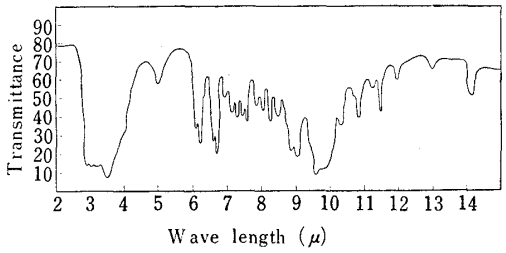

Fig. 1. Infrared spectrum of Pseudoneamine Trihydrochloride

From the filtrate from pseudoneamine trihydrochloride, amorphous methyl zygobiosaminide, a moiety of the methylglycoside molecule, was obtained by fractional precipitation and purification by chromatography on alumina or carbon. It was positive to Ninhydrin reaction but negative to Elson-Morgan and aniline hydrogenphthalate tests.

Hydrolysis of methyl zygobiosaminide with dilute hydrochloric acid for 2.5 hours at 100° produced zygobiosamine, which afforded D-ribose and diaminohexose[4] on further hydrolysis.

This disaccharide gave positive Fehling and aniline hydrogenphthalate reactions.

Methyl zygobiosaminide was N-acetylated by the mothod of Roseman and Ludowieg,[7] affording amorphous methyl N,N′-diacetylzygobiosaminide, $C_{11}H_{19}O_7N_2(OCH_3)(COCH_3)_2$.

The product was hydrolyzed with dilute sulfuric acid and the treatment of the reaction mixture with barium carbonate, followed by chromatography on ion exchange resin and carbon, afforded a crystalline neutral carbohydrate, which was in accord with D-ribose in color test, papergram, specific rotation, and infrared absorption spectrum.

Identity of the carbohydrate with D-ribose was also afforded by the fact that it gave crystalline ribitol, m.p. 98~100°, by reduction with sodium borohydride.

Experimental

Isolation of Pseudoneamine Trihydrochloride——A solution of 5 g. of zygomycin-A hydrochloride in 650 cc. of 0.3*N* MeOH-HCl was refluxed for 3 hr., during which a crystalline precipitate deposited. After refrigerating overnight, the precipitate consisting of the trihydrochloride of pseudoneamine was collected by filtration and purified by recrystallization from hydr. EtOH, m.p. 232° (decomp.). $[\alpha]_D^{22}$ +83° (c=0.5, H_2O). *Anal.* Calcd. for $C_{12}H_{25}O_7N_3 \cdot 3HCl \cdot H_2O$: C, 31.98; H, 6.71; N, 9.32. Found: C, 31.70; H, 6.66; N, 9.28.

Preparation of Free Base of Pseudoneamine——A solution of pseudoneamine trihydrochloride was passed through a column of Dowex 1 (OH^- form). The effluent and washings were concentrated *in vacuo*, and EtOH was added to the residue until the solution became cloudy. This was allowed to cool in an ice box, affording the crystalline free base of pseudoneamine, m.p. 250~251° (decomp.). $[\alpha]_D^{22}$ +124° (c=1, H_2O). *Anal.* Calcd. for $C_{12}H_{25}O_7N_3 \cdot \frac{1}{2}H_2O$: C, 43.36; H, 7.88; N, 12.64. Found : C, 43.69; H, 8.13; N, 12.93.

Preparation of N,N′,N″-Triacetylpseudoneamine——Pseudoneamine trihydrochloride was N-acetylated by the method of Roseman and Ludowieg.[7] The product was crystallized from AcOH, m.p.

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6) K. Maeda, M. Murase, H. Mawatari, H. Umezawa : J. Antibiotics (Japan), **11A**, 73 (1958).
7) S. Roseman, J. Ludowieg : J. Am. Chem. Soc., **76**, 301 (1954).

300~305° (decomp.). *Anal.* Calcd. for $C_{12}H_{22}O_7N_3(CH_3CO)_3 \cdot CH_3COOH$: C, 47.15; H, 6.92; N, 8.25. Found : C, 47.23; H, 6.90; N, 8.72.

Isolation of Deoxystreptamine Dihydrochloride——Pseudoneamine trihydrochloride (1 g.) was dissolved in 8.5 cc. of conc. HCl and the solution was refluxed for 2.5 hr. The hydrolysate was concentrated *in vacuo* and Me_2CO was added to the residue, affording a brown amorphous powder (0.99 g.). A solution of this material in water (4 cc.) was treated with carbon and, after addition of EtOH (25 cc.), allowed to cool in an ice box. The resulting white crystals were collected by filtration and recrystallized from hydr. EtOH. The infrared absorption spectrum and X-ray diffraction pattern of the product were identical with those of deoxystreptamine, m.p. 325° (decomp.), $[\alpha]_D^{22}$ 0° (c=0.5, H_2O), derived from kanamycin. *Anal.* Calcd. for $C_6H_{14}O_3N_2 \cdot 2HCl$: C, 30.65; H, 6.86; N, 11.92. Found: C, 30.61; H, 6.83; N, 12.00.

Isolation of D-Glucosamine Hydrochloride——To the mother liquor from deoxystreptamine hydrochloride, 10 cc. of MeOH and 25 cc. of Me_2CO were added. The resulting precipitate was removed by filtration and to the filtrate were added 50 cc. of Me_2CO and 50 cc. of Et_2O, affording crystalline D-glucosamine hydrochloride. It was recrystallized from hydr. EtOH. The infrared spectrum and X-ray diffraction pattern of the product were identical with those of authentic D-glucosamine hydrochloride, m.p. 188~209° (decomp.). $[\alpha]_D^{21}$ +89°→+72° (c=1, H_2O). *Anal.* Calcd. for $C_6H_{13}O_5N \cdot HCl$: C, 33.42; H, 6.56; N, 6.50. Found : C, 33.25; H, 6.67; N, 6.71.

Isolation of Methyl Zygobiosaminide Dihydrochloride——The mother liquor from pseudoneamine trihydrochloride was concentrated *in vacuo* and the concentrate was subjected to fractional precipitation with Et_2O, affording a white amorphous powder.

The product (1.47 g.) was purified by dissolving it in MeOH-Et_2O mixture (2:1) (150 cc.) and passing the solution through a column of alumina (50 g.), followed by eluting with a MeOH-Et_2O mixture (5:2). The methyl zygobiosaminide thus purified was collected by evaporating the effluent to a small amount *in vacuo* and adding Et_2O to the residue. $[\alpha]_D^{21}$ +29° (c=1.0, H_2O).

Preparation of Methyl N,N'-Diacetylzygobiosaminide——Methyl zygobiosaminide dihydrochloride was N-acetylated by the method of Roseman and Ludowieg,[7] affording the desired product as a hygroscopic amorphous powder. *Anal.* Calcd. for $C_{11}H_{19}O_7N_2(COCH_3)_2(OCH_3)$: C, 47.11; H, 6.91; N, 6.86. Found : C, 46.70; H, 6.99; N, 6.68.

Isolation of D-Ribose from Methyl N,N'-Diacetylzigobiosaminide——A solution of methyl N,N'-diacetylzygobiosaminide in 0.1*N* H_2SO_4 was heated under reflux for 3.5 hr. After cool, the hydrolysate was neutralized with $BaCO_3$ and the resulting precipitate was collected and washed. The filtrate and washings were combined and passed successively through columns of Amberlite IR-120 (H^+ form) and IRA-400 (OH^- form). The effluent and washings were concentrated *in vacuo* and the concentrate was passed through a column of charcoal. The Fehling test-positive fraction was collected, concentrated to a syrup *in vacuo*, and EtOH was added. The mixture was allowed to stand in an ice box, affording white crystals, m.p. 86°. The infrared spectrum of the product was identical with that of D-ribose and it showed no depression in melting point on admixture with an authentic sample.

Since the sugar exhibited a specific rotation, $[\alpha]_D^{22}$ −14°→−17° (24 hr.) (c=1.0, H_2O), it was assumed to belong to the D-series.

Reduction of D-Ribose with Sodium Borohydride——The colorless syrup prepared by the procedure mentioned above was reduced with $NaBH_4$ and the reaction mixture was passed succcessively through columns of Amberlite IR-120 (H^+ form) and Amberlite IRA-400 (OH^- form) to remove inorganic impurities. The effluent and washings were evaporated to a syrup, which was allowed to stand in an ice box with addition of EtOH, affording white crystals, m.p. 98~100°. The infrared spectrum of the product was identical with that of ribitol and there was observed no depression in melting point on admixture with an authentic sample. *Anal.* Calcd. for $C_5H_{12}O_5$: C, 39.47; H, 7.95. Found : C, 39.93; H, 7.93.

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Summary

Zygomycin-A, when subjected to methanolysis, produced a crystalline pseudoneamine trihydrochloride and amorphous methyl zygobiosaminide dihydrochloride ($C_{11}H_{21}O_7N_2(OCH_3) \cdot 2HCl$). Methyl-N,N'-diacetylzygobiosaminide on hydrolysis yielded D-ribose.

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